

APPLICANTS: Peled, *et al.*  
SERIAL NUMBER: 10/767,064

**Listing of the Claims:**

This listing of claims will replace all prior versions and listing of claims in the application:

1-200. (Cancelled)

201. (Currently amended) A method of expanding an *ex-vivo* population of CD34+, CD34+/CD38- and CD 133+ hematopoietic stem cells, while at the same time, inhibiting differentiation of the hematopoietic stem cells *ex-vivo*, the method comprising

(a) providing hematopoietic mononuclear cells that are not enriched prior to culturing;

(b) culturing said mononuclear cells *ex-vivo* for a period of at least 14 ~~greater than 7~~ days under conditions allowing for cell proliferation, said conditions comprising:

(i) providing nutrients and ~~at least an early acting cytokine or a combination of~~ cytokines selected from the group consisting of thrombopoietin (TPO), interleukin 6 (IL-6), FLT-3 ligand, stem cell factor (SCF) and interleukin 3 (IL-3);

(ii) removing one half of the culture volume and replacing it with fresh medium and cytokines weekly;

and, at the same time, culturing said mononuclear cells under conditions inhibiting differentiation of said hematopoietic stem cells, said conditions comprising providing an amount of tetraethylenepentamine (TEPA) at least one copper chelator which reduces effective in reducing intracellular available copper concentration in said cells;

thereby expanding a population of said hematopoietic stem cells while at the same time inhibiting differentiation of said hematopoietic stem cells *ex-vivo* for a period of at least 14 ~~greater than 7~~ days.

202-208. (Cancelled)

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209. (Original) The method of claim 201, wherein said hematopoietic mononuclear cells are derived from a source selected from the group consisting of bone marrow, peripheral blood and neonatal umbilical cord blood.

210-211. (Cancelled)

212. (Currently Amended) The method of claim 201, wherein said ~~early-acting cytokine or cytokines is selected from the group consisting of~~ combination of cytokines comprises thrombopoietin (TPO), interleukin 6 (IL-6), stem cell factor (SCF), FLT3 ligand, interleukin-1, interleukin-2, interleukin-3, interleukin-6 (IL-6), interleukin-10, interleukin-12, tumor-necrosis-factor- $\alpha$  and thrombopoietin (TPO).

213. (Previously presented) The method of claim 201, further comprising providing a late acting cytokine or cytokines.

214. (Previously presented) The method of claim 213, wherein said late acting cytokine or cytokines is selected from the group consisting of granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor, erythropoietin, FGF, EGF, NGF, VEGF, LIF, Hepatocyte growth factor and macrophage colony stimulating factor.

215-238. (Canceled)

239. (Previously presented) The method of claim 201, wherein said hematopoietic mononuclear cells comprise a major fraction of hematopoietic committed cells and a minor fraction of hematopoietic stem and progenitor cells.

240-243. (Canceled)

244. (Currently Amended) The method of claim 201, wherein said amount of at least

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~~one copper chelator~~ is tetraethylenepentamine (TEPA) is 0.5 to 20.0  $\mu$ M.

245. (Currently Amended) The method of claim ~~201~~244, wherein said amount of tetraethylenepentamine (TEPA) is 5.0  $\mu$ M ~~period is greater than 14 days.~~

246. (New) The method of claim 201, wherein said period is greater than 5 weeks.

247. (New) A method of expanding an *ex-vivo* population of CD34+, CD34+/CD38- and CD 133+ hematopoietic stem cells, while at the same time, inhibiting differentiation of the hematopoietic stem cells *ex-vivo*, the method comprising

- (a) providing hematopoietic mononuclear cells that are not enriched prior to culturing;
- (b) culturing said mononuclear cells *ex-vivo* for a period greater than 14 days under conditions allowing for cell proliferation, said conditions comprising:
  - (i) providing nutrients and a combination of early acting cytokines selected allowing proliferation of hematopoietic stem cells;
  - (ii) removing one half of the culture volume and replacing it with fresh medium and cytokines weekly;

and, at the same time, culturing said mononuclear cells under conditions inhibiting differentiation of said hematopoietic stem cells, said conditions comprising providing an amount of tetraethylenepentamine (TEPA) effective in reducing intracellular available copper concentration in said cells;

thereby expanding a population of said hematopoietic stem cells while at the same time inhibiting differentiation of said hematopoietic stem cells *ex-vivo* for a period greater than 14 days.

248. (New) The method of claim 247, wherein said combination of early acting cytokines is selected from the group consisting of interleukin 6 (IL-6), stem cell factor (SCF), FLT3 ligand, interleukin-1, interleukin-2, interleukin-3, interleukin-6 (IL-6), interleukin-10,

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interleukin-12, tumor necrosis factor- $\alpha$  and thrombopoietin(TPO).